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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/758,875	01/16/2004	Michael L. Weiner	034405-013	6570

21839 7590 07/12/2006

BUCHANAN, INGERSOLL & ROONEY PC
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EXAMINER	
BERTAGNA, ANGELA MARIE	
ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 07/12/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/758,875	WEINER ET AL.	
	Examiner	Art Unit	
	Angela Bertagna	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 May 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-63* is/are pending in the application.

4a) Of the above claim(s) *9-31 and 47-63* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-8 and 32-46* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *16 January 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *3/25/2004*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election without traverse of Group I, claims 1-8 and 32-46, in the reply filed on May 11, 2006 is acknowledged.

Claims 9-31 and 47-63 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on May 11, 2006.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Priority

2. Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged. Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(e) as follows:

The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35

U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

The disclosure of the prior-filed application, Application No. 60/184,120, filed 2/18/2000, fails to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for one or more claims of this application. This provisional application appears to be directed to computational methods for analyzing nucleic acids, and does not describe the nanoparticle-based nucleic acid assemblies of claims 1-8 or the dendrimer detection complexes of claims 32-46 of the instant application. The only mention of nanostructures appears on page 58 and does not provide adequate support for the instant claims. Therefore, the instant application has not been granted benefit of the earlier filing date of the above provisional application, and an effective filing date of February 17, 2001 (filing date of PCT/US01/05139) has been used.

Specification

3. Applicant is reminded of the proper language and format for an abstract of the disclosure.

The abstract should be in narrative form and generally limited to a single paragraph on a separate sheet within the range of 50 to 150 words. It is important that the abstract not exceed 150 words in length since the space provided for the abstract on the computer tape used by the printer is limited. The form and legal phraseology often used in patent claims, such as "means" and "said," should be avoided. The abstract should describe the disclosure sufficiently to assist readers in deciding whether there is a need for consulting the full patent text for details.

The language should be clear and concise and should not repeat information given in the title. It should avoid using phrases which can be implied, such as, "The disclosure concerns," "The disclosure defined by this invention," "The disclosure describes," etc.

Specifically, the abstract uses the legal phraseology "means", for example, "means for introducing energy into the first nanoparticle."
Appropriate correction is required.

Claim Rejections - 35 USC § 102

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

5. Claims 1 and 3-5 are rejected under 35 U.S.C. 102(b) as being anticipated by Mirkin et al. (Nature (1996) 382: 607-609).

Regarding claim 1, Mirkin teaches a detection device comprised of a hybrid nucleic acid assembly (Figure 1). The hybrid nucleic acid assembly taught in Figure 1 of Mirkin comprises a nucleic acid polymer and a first and second nanoparticle conjugated to the nucleic acid polymer. Specifically, the first and second nanoparticles are conjugated to the nucleic acid polymer via the "linking DNA duplex". This double-stranded DNA molecule has two "sticky ends" that hybridize to the oligonucleotides immobilized on the two nanoparticles, thereby conjugating the nanoparticles (see Figure 1 and also page 608). Note that the specification does not require that the conjugation be covalent, and therefore, the assembly of Mirkin is a conjugated system. The oligonucleotide-functionalized gold nanoparticles of Mirkin comprise a means for introducing energy into the first nanoparticle and a means for detecting energy from the second nanoparticle, because upon conjugation of first and second nanoparticles via hybridization with the duplex DNA, an absorbance transition (red to purple) occurs (see page 608 and Figure 2). Therefore,

the nanoparticle-DNA hybrid assembly contains (within itself) a means for introducing energy (light) and detecting energy. Finally, the hybrid assembly taught by Mirkin includes a means for determining a physical property of the nucleic acid polymer, because since the above color change only occurs upon hybridization of the duplex linker to the complementary nanoparticle-immobilized oligonucleotides, observation of the color change is indicative of a physical property of the nucleic acid polymer, namely that it has hybridized to the functionalized nanoparticles.

Regarding claims 3 and 4, Mirkin teaches gold nanoparticles (see Figure 1 and page 607, column 2).

Regarding claim 5, formation of the assembly taught by Mirkin produces a mechanical property (a change in the flexibility of the duplex linker DNA). The images acquired using transmission electron microscopy (TEM) of the nucleic acid assembly provided information about this mechanical property of the nucleic acid polymer (page 608, column 2).

6. Claims 1 and 3-5 are rejected under 35 U.S.C. 102(e) as being anticipated by Bamdad (US 2002/0098526 A1).

Regarding claim 1, Bamdad teaches a detection device comprising a hybrid nucleic acid assembly (paragraph 6 and Figure 1). The hybrid assembly comprises a nucleic acid polymer conjugated to a first and second nanoparticle (see Figures 5 & 6 and paragraphs 90-91, or alternatively, Example 2 on page 13). Bamdad also teaches covalent attachment mechanisms (paragraph 59). The hybrid assembly of Bamdad further comprises a means for introducing energy to the first nanoparticle and means for detecting energy from the second nanoparticle,

because upon conjugation of first and second nanoparticles via hybridization of the complementary DNA strands, an absorbance transition (red to purple) occurs (paragraphs 112-113). Therefore, the nanoparticle-DNA hybrid assembly contains (within itself) a means for introducing energy (light) and detecting energy. Finally, the hybrid assembly taught by Bamdad includes a means for determining a physical property of the nucleic acid polymer, because since the above color change only occurs upon hybridization of complementary DNA nanoparticle-immobilized DNA strands, observation of the color change is indicative of a physical property of the nucleic acid polymer, namely that it has hybridized to the functionalized nanoparticles.

Regarding claims 3 and 4, Bamdad teaches that the first and second nanoparticles may be gold (Example 2, paragraphs 109-113).

Regarding claim 5, Bamdad teaches that the assembly may function as a biosensor, where changes in mechanical properties such as flow rate, pressure and/or electroosmotic forces are measured and related to a physical property of the nucleic acid polymer, namely its ability to hybridize to a test sample (paragraphs 84-87, in particular paragraphs 86-87).

7. Claims 1, 3, and 5 are rejected under 35 U.S.C. 102(e) as being anticipated by Dubertret et al. (US 2004/0002089 A1).

Regarding claims 1 and 3, Dubertret teaches a detection device comprising a hybrid nucleic acid assembly (see abstract and Figure 1b). The hybrid nucleic acid assembly of Dubertret comprises a nucleic acid polymer covalently linked to two nanoparticles (see Figure 1b). Dubertret teaches that the first nanoparticle may be a gold nanoparticle (Figure 1b; paragraph 10) and that the second nanoparticle may be a luminescent quantum dot (Figure 1b;

paragraph 12). The hybrid assembly of Dubertret includes a means for introducing energy to the first nanoparticle and a means for detecting energy from the second nanoparticle (paragraphs 17 & 38 teach applying fluorescence energy to both nanoparticles and detection of the change in the signal emitted from the second particle). The information gained by the change in fluorescence of the second nanoparticle provides a means of determining a physical property of the nucleic acid polymer, specifically its ability to hybridize with an added binding partner (paragraphs 17 and 38).

Regarding claim 5, the hybrid assembly of Dubertret includes a means for producing a mechanical property (specifically, conformational changes that inherently lead to increased flexibility of the hybrid assembly upon binding to a provided target; see paragraph 38) that provides information about the physical property of the nucleic acid polymer, specifically its hybridization state.

8. Claims 32-36, and 38-45 are rejected under 35 U.S.C. 102(b) as being anticipated by Tomalia et al. (USPN 5,714,166).

Regarding claim 32, Tomalia teaches a dendrimer-nucleic acid-energy detector complex (column 1, line 66 – column 2, line 9) comprising a nucleic acid sequence and a dendrimer (column 2, lines 10-26). Tomalia also teaches that the dendrimer-nucleic acid complex may further include a signal generator, such as a fluorescent, bioluminescent, or phosphorescent entity, a signal reflector, such as a paramagnetic entity, or a signal absorber, such as a dye (column 16, line 56-column 17, line 33), all of which are energy detection devices.

Regarding claims 33 and 36, Tomalia teaches that the dendrimer comprises a conductive material, specifically metalloids (column 12, lines 42-45).

Regarding claim 34, Tomalia teaches that the dendrimer has a star shape, a starburst shape (Figure 1; column 1, lines 45-50), a spherical shape, or rod shape (column 12, lines 1-10).

Regarding claim 35, Tomalia teaches that the energy detection device is a fluorophore or chromophore (column 16, line 56 – column 17, line 33; see especially column 16, lines 59-62).

Regarding claim 38, Tomalia teaches that the complex is incorporated into an encapsulating agent (column 28, lines 53-63).

Regarding claims 39-41, Tomalia teaches transfection of the complex into a cultured cell (see column 41, lines 18-23 and also Example 70, column 120, lines 33-52) or administration to a multi-cellular organism (column 40, lines 35-46).

Regarding claims 42-45, Tomalia teaches that the nucleic acid may be DNA (single or double-stranded), RNA (which is a single stranded nucleic acid) (column 2, lines 16-27).

9. Claims 32-37 and 39-46 are rejected under 35 U.S.C. 102(e) as being anticipated by Tomalia et al. (USPN 6,475,994 B2).

Regarding claim 32, Tomalia teaches a dendrimer-nucleic acid-energy detector complex (column 2, lines 19-24) comprising a nucleic acid sequence (column 3, lines 1-7), a dendrimer (column 3, lines 9-10), and an energy detector (column 2, line 21 and lines 38-41; where the metals are energy detectors).

Regarding claims 33 and 36, the dendrimer of Tomalia comprises a conductive polymer, specifically a metal, such as gold or copper (column 2, lines 38-41).

Regarding claim 34, Tomalia teaches that the dendrimer may have a spherical or rod shape (column 4, line 67 – column 5, line 1). Tomalia also teaches the use of star shaped dendrimers (column 6, line 15).

Regarding claim 35, the conductive gold metal component of the dendrimer complex taught by Tomalia is inherently capable of transmitting and receiving energy. Therefore, this energy detection device may function as a transceiver.

Regarding claim 37, Tomalia teaches that the dendrimer polymer may be coated with gold nanoparticles (column 8, lines 44-52; where the gold particles in the gold sol are nanoparticles since their maximum dimension may be from 1-100 nm).

Regarding claims 39-41, Tomalia teaches transfection of the complex into cultured cells or administration to a multi-cellular organism (column 8, line 59 – column 9, line 3).

Regarding claims 42-46, Tomalia teaches that the nucleic acid may be DNA (a double-stranded nucleic acid), RNA (which is a single stranded nucleic acid), or cDNA (column 3, lines 1-8).

Claim Rejections - 35 USC § 103

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various

claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

11. Claims 2 and 6-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Unger et al. (USPN 6,443,898 B1) in view of either of Mirkin et al. (Nature, 1996) or Dubertret et al. (US 2004/0002809 A1).

Unger teaches therapeutic delivery systems comprising a lipid-encapusulated target agent (including nucleic acids) (see abstract and column 25, lines 42-52). Unger further teaches the inclusion of nanoparticles with the encapsulated therapeutic agent (column 23, lines 33-40).

Regarding claim 2, Unger teaches the application of ultrasonic energy to the therapeutic delivery system to release the contents of the liposome (column 28, lines 35-38; see also column 31, line 58 – column 32, line 3).

Regarding claims 6-8, Unger teaches transfection into cultured cells (column 33, lines 38-42) and also administration to multi-cellular organisms (column 33, lines 43-53).

Unger does not explicitly teach that the therapeutic agent (antisense nucleic acid) is conjugated to the nanoparticles.

Mirkin and Dubertret separately teach the device of claim 1, as discussed above.

Neither Mirkin nor Dubertret teach application of ultrasonic energy to the nanoparticle conjugate or transfection of the device into a cell.

It would have been prima facie obvious for one of ordinary skill in the art at the time of invention to incorporate the oligonucleotide-nanoparticle assemblies taught by either Mirkin or Dubertret into the therapeutic delivery systems of Unger in order to improve the detection capability of the system. Unger teaches monitoring the efficacy of the therapy solely through the use of ultrasound (see for example, column 4, lines 48-51). Mirkin and Dubertret separately taught visual (colorimetric) detection of nanoparticle-oligonculeotide conjugates where the detectable signal resulted from hybridization to a complementary nucleic acid (see page 608 of Mirkin and paragraphs 17 and 38 of Dubertret). The person of ordinary skill would have been motivated to utilize these directly detectable nanoparticle-nucleic acid conjugates to quickly and easily monitor their binding to target nucleic acids. The person of ordinary skill would have been particularly motivated to use the oligonucleotide-nanoparticle conjugates of Mirkin or Dubertret in the critical early-stage in vitro cell transfection studies proposed by Unger (column 33, lines 38-42), because doing so would have provided a rapid and direct method of detecting binding between the released oligonucleotide-nanoparticle conjugates the target nucleic acid, thereby minimizing the time required for screening multiple targets and reaction conditions. Therefore, the person of ordinary skill, interested in rapidly and easily detecting binding between a potential therapeutic nucleic acid and its target, would have been motivated to use the directly detectable oligonucleotide-nanoparticle conjugates taught by either Mirkin or Dubertret, thus resulting in the instantly claimed invention.

Conclusion

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Torimoto et al. (Journal of Physical Chemistry B (1999) 42(103): 8799-8803) and Coffert et al. (Nanotechnology (1992) 3:69-76) teach attachment of multiple CdS nanoparticles to an immobilized DNA molecule. Baker et al. (USPN 6,471,968 B1) also teaches nucleic acid-dendrimer-energy detection devices.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Angela Bertagna whose telephone number is (571) 272-8291. The examiner can normally be reached on M-F 7:30-5 pm EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Angela Bertagna
Patent Examiner
Art Unit 1637

amb

JEFFREY FREDMAN
PRIMARY EXAMINER

PTO/SB/08A (04-03)
Approved for use through 04/30/2003. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 8

Complete if Known	
Application Number	10/758,875
Filing Date	1/16/2004
First Named Inventor	Weiner, Michael L.
Art Unit	Unknown
Examiner Name	Unknown
Attorney Docket Number	MLW-630

U. S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
AB		US- 6,180,385	01/30/2001	Skern et al.	
		US- 6,180,389	01/30/2001	Douglas et al.	
		US- 6,180,402	1/30/2001	Granville et al.	
		US- 6,180,612	01/30/2001	Hockensmith et al.	
		US- 6,180,782	01/30/2001	Capuder	
		US- 6,183,984	02/06/2001	Fuchs	
		US- 6,183,993	02/06/2001	Boyce et al.	
		US- 6,183,174	02/06/2001	Kaiser et al.	
		US- 6,183,753	02/06/2001	Cochran et al.	
		US- 6,184,000	02/06/2001	Jones et al.	
		US- 6,184,017	02/06/2001	Smith et al.	
		US- 6,184,027	02/06/2001	Laine et al.	
		US- 6,184,034	02/06/2001	Eastman et al.	
		US- 6,187,250	02/13/2001	Champagne	
		US- 6,187,552	02/13/2001	Roberds et al.	
		US- 6,187,559	02/13/2001	Steed et al.	
		US- 6,187,564	02/13/2001	Sytkowski	
		US- 6,187,566	02/13/2001	Dattagupta et al.	
AB		US- 6,187,567	02/13/2001	Li et al.	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]-Number[4]-Kind Code[5] (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T[6]

Examiner Signature	/Angela Bertagna/	Date Considered	05/23/2006

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

PTO/SB/08A (04-03)
Approved for use through 04/30/2003. OMB 0651-0031
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Sheet 2 of 8

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AB		US- 6,187,567	02/13/2001	Li et al.	
		US- 6,187,575	02/13/2001	Sobek et al.	
		US- 6,187,578	02/13/2001	Blinkovsky et al.	
		US- 6,187,585	02/13/2001	Bennett et al.	
		US- 6,187,760	02/13/2001	Ashley et al.	
		US- 6,187,823	02/13/2001	Haddon et al.	
		US- 6,203,814	03/20/2001	Fisher et al.	
		US- 6,331,262	12/18/2001	Haddon et al.	
		US- 6,399,365	06/04/2002	Besemer et al.	
		US- 6,413,722	07/02/2002	Arnold et al.	
		US- 6,471,968	10/29/2002	Baker Jr. et al.	
		US- 6,475,994	11/05/2002	Tomalia et al.	
		US- 6,618,941	09/16/2003	Campbell et al.	
		US- 6,013,789	01/11/2000	Rampal	
		US- 6,017,731	01/25/2000	Tekamp-Olson et al.	
		US- 6,020,457	02/01/2000	Klimash et al.	
		US- 5,872,826	02/16/1999	Fujieda et al.	
		US- 6,043,336	03/28/2000	Miller et al.	
AB		US- 6,051,379	04/18/2000	Lescallett et al.	

FOREIGN PATENT DOCUMENTS

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Examiner Signature	/Angela Bertagna/	Date Considered	05/23/2006

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Sheet 3 of 8

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AB		US- 6,054,708	04/25/2000	Shimizu	
		US- 6,083,698	07/04/2000	Olson et al.	
		US- 6,083,708	07/04/2000	Singh et al.	
		US- 6,083,723	07/04/2000	Tekamp-Olson	
		US- 6,136,318	10/24/2000	Cochran et al.	
		US- 5,776,672	07/07/1998	Hashimoto et al.	
		US- 5,972,604	10/26/1999	Santamaria et al.	
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Sheet 6 of 8

Complete if Known	
Application Number	10/758,875
Filing Date	1/16/2004
First Named Inventor	Weiner, Michael L.
Art Unit	Unknown
Examiner Name	Unknown
Attorney Docket Number	MLW-630

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Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[2]
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Sheet 7 of 8

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First Named Inventor	Weiner, Michael L.
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Examiner Name	Unknown
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A DNA-based method for rationally assembling nanoparticles into macroscopic materials

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Department of Chemistry, Northwestern University, Evanston, Illinois 60208, USA

Colloidal particles of metals and semiconductors have potentially useful optical, optoelectronic and material properties[1-4] that derive from their small (nanoscopic) size. These properties might lead to applications including chemical sensors, spectroscopic enhancers, quantum dot and nanostructure fabrication, and microimaging methods[2-4]. A great deal of control can now be exercised over the chemical composition, size and polydispersity[1,2] of colloidal particles, and many methods have been developed for assembling them into useful aggregates and materials. Here we describe a method for assembling colloidal gold nanoparticles rationally and reversibly into macroscopic aggregates. The method involves attaching to the surfaces of two batches of 13-nm gold particles non-complementary DNA oligonucleotides capped with thiol groups, which bind to gold. When we add to the solution an oligonucleotide duplex with 'sticky ends' that are complementary to the two grafted sequences, the nanoparticles self-assemble into aggregates. This assembly process can be reversed by thermal denaturation. This strategy should now make it possible to tailor the optical, electronic and structural properties of the colloidal aggregates by using the specificity of DNA interactions to direct the interactions between particles of different size and composition.

Previous assembly methods have focused on the use of covalent 'linker' molecules that possess functionalities at opposing ends with chemical affinities for the colloids of interest. One of the most successful approaches to date[5] has involved the use of gold colloids and well established thiol adsorption chemistry[6,7]. In this approach, linear alkanedithiols were used as the particle linker molecules. The thiol groups at each end of the linker molecule covalently attach themselves to the colloidal particles to form aggregate structures. The drawbacks of this method are that the process is difficult to control and the assemblies are formed irreversibly. Methods for systematically controlling the assembly process are needed if the materials properties of these unusual structures are to be exploited fully.

Our oligonucleotide-based method allows the controlled and reversible assembly of gold nanoparticles into supramolecular structures. Oligonucleotides offer several advantages over non-biological-based linker molecules. For example, discrete sequences of controlled length and with the appropriate surface binding functionality may be prepared in an automated fashion with a DNA synthesizer. In this way, the molecular recognition properties of the oligonucleotides may be used to trigger the colloidal self-assembly process. The interparticle distances and stabilities of the supramolecular structures generated by this method can be controlled through the choice of oligonucleotide sequence and length, solvent, temperature and supporting electrolyte concentration.

Others also have recognized the utility of DNA for the preparation of new biomaterials and nanofabrication methods. Previous researchers have focused on using the sequence-specific molecular-recognition properties of oligonucleotides to design impressive structures with well defined geometric shapes and sizes[8-18]. The chemistry proposed here focuses on merging the chemistry of DNA with the chemistry of inorganic colloidal materials. In addition to generating materials with properties that are hybrids of their DNA and colloidal precursors, the union of metal-colloid and DNA chemistry offers significant opportunities relative to the construction of pure DNA materials. As noted by Seeman[19], 'the theory of producing DNA [structures] is well ahead of experimental confirmation. It is much easier to design a [structure] than it is to prove its synthesis.' An advantage of the DNA/colloid hybrid materials reported herein is that the assemblies can be characterized easily by transmission electron microscopy (TEM) and/or atomic force microscopy (AFM) as well as spectroscopic methods conventionally used with DNA.

Our approach to using oligonucleotides for the controlled assembly of gold nanoparticles into aggregate macroscopic structures is outlined in Fig. 1. First, 13-nm-diameter Au particles are prepared[2,20]. These particles form a dark red suspension in water, and like thin-film Au substrates[21], they are easily modified with oligonucleotides, which are functionalized with alkane thiols at their 3′ termini. In a typical experiment, one solution of 17 nM (150 μl) Au colloids is treated for 24 h with 3.75 μM (46 μl) 3′-thiol-TTTGCTGA, and a second solution of colloids is treated with 3.75 μM (46 μl) 3′-thiol-TACCGTTG. Note that these oligonucleotides are non-complementary. After treatment with the thiol-capped oligonucleotides, the two colloidal Au solutions are combined, and because of the non-complementary nature of the oligonucleotides, no reaction takes place. A beneficial consequence of capping the colloids with these oligonucleotides is

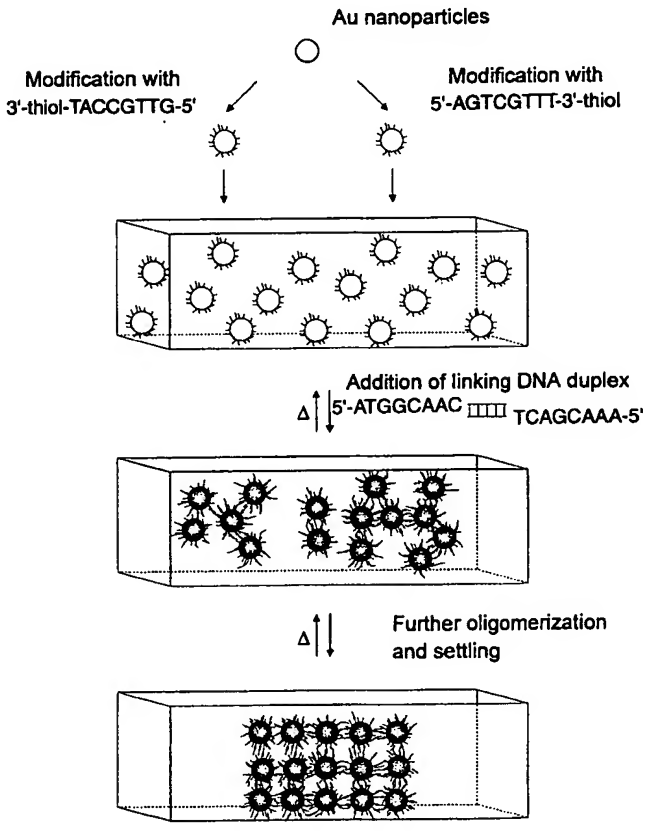

FIG. 1 Scheme showing the DNA-based colloidal nanoparticle assembly strategy (the hybridized 12-base-pair portion of the linking duplex is abbreviated as). If a duplex with a 12-base-pair overlap but with 'sticky ends' with four base mismatches (5′-AAGTCAGTTATACGCGCTAG and 3′-ATATGCGCGATCAAATCACA) is used in the second step, no reversible particle aggregation is observed. The scheme is not meant to imply the formation of a crystalline lattice but rather an aggregate structure that can be reversibly annealed. Δ is the heating above the dissociation temperature of the duplex.

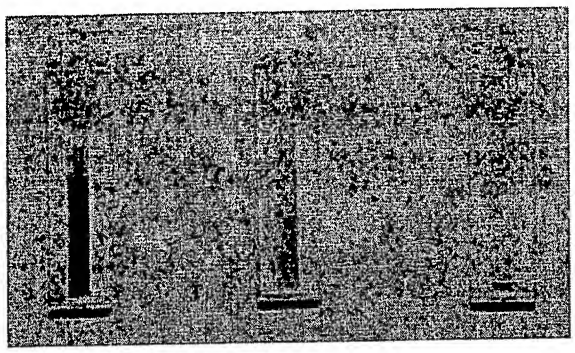

FIG. 2 Cuvettes with the Au colloids and the four DNA strands responsible for the assembly process. Left cuvette, at 80 °C with DNA-modified colloids in the unhybridized state; centre, after cooling to room temperature but before the precipitate settles; and right, after the polymeric precipitate settles to the bottom of the cuvette. Heating either of these cool solutions results in the reformation of the DNA-modified colloids in the unhybridized state (shown in the left cuvette).

that they are much more stable than bare Au colloids to increased salt concentration and temperature. When heated or in a solution of high salt concentration (0.1 M NaCl), bare colloids undergo irreversible particle-growth reactions that result in their precipitation. In contrast, the DNA-modified Au nanoparticles reported here are stable at elevated temperatures (80 °C) and in aqueous 0.1 M NaCl solutions for days, presumably because their DNA-modified surfaces prohibit them from getting close enough to undergo particle growth. This is important because high salt concentrations are needed for the DNA hybridization events depicted in Fig. 1.

In the next step of the assembly scheme, a duplex consisting of 5′-ATGGCAACTATACGCGCTAG and 3′-ATATGCGCGA-TCTCAGCAAA (the duplex has a 12-base-pair overlap (underlined), containing 8-base-pair sticky ends, which are complementary to the 8-base-pair oligonucleotides that are covalently attached to the Au colloids; Fig. 1) is added to the dark red solution. The solution is then diluted with aqueous NaCl (to 1 M) and buffered at pH 7 with 10 mM phosphate, conditions which are suitable for hybridization of the oligonucleotides. Significantly, an immediate colour change from red to purple is observed and a precipitation reaction ensues. Over the course of several hours, the solution becomes clear and a pinkish-grey precipitate settles to the bottom of the reaction vessel (Fig. 2). Presumably, the free ends of the 'linking' duplex bind to the complementary oligomers anchored to the gold, thereby crosslinking the colloids, which ultimately results in the formation of the pinkish-grey polymeric DNA–colloid precipitate. To verify that this process involved both the DNA and colloids, the precipitate was collected and resuspended (by shaking) in 1 M aqueous NaCl buffered at pH 7. Then, a temperature/time dissociation experiment was performed by monitoring both an optical absorption dependent on hybridization of DNA (260 nm) and one dependent on the degree of colloid aggregation (700 nm), Fig. 3*a*. As the temperature is cycled between 0 and 80 °C, which is 38 °C above the dissociation temperature (T_m) for the DNA-duplex (T_m = 42 °C), there is an excellent correlation between the optical signatures for both the colloids and DNA. In the absence of DNA, the ultraviolet–visible spectrum for the naked Au colloids is much less temperature-dependent (Fig. 3*b*). There is a substantial optical change when the polymeric DNA–colloid precipitate is heated above its melting point. The clear solution turns dark red as the polymeric biomaterial dehybridizes to generate the unlinked colloids which are soluble in the aqueous solution. This process is very reversible as evidenced by the temperature traces in Fig. 3*a*. In a control experiment designed to verify that this process was due to oligonucleotide hybridization, a duplex with four base-pair mismatches in each of the 'sticky' ends of the linkers (step 2 in Fig. 1) did not induce the reversible particle aggregation process.

Further evidence of the polymerization/assembly process comes from TEM studies of the polymeric precipitate (Fig. 4). TEM images of the colloids linked with hybridized DNA show large assembled networks of the Au colloids (Fig. 4*a*). Naked Au colloids do not aggregate in this manner under comparable conditions, but rather undergo particle-growth reactions[2]. Note that there is no evidence of colloid particle growth as the hybridized colloids seem to be remarkably regular in size with an average diameter of 13 nm. With TEM, because of the superposition of layers, it is difficult to assess the degree of order for three-dimensional aggregates. But smaller-scale images of single-layer, two-dimensional aggregates provide more compelling evidence of the self-assembly process (Fig. 4*b*). This figure shows close-packed assemblies of the aggregates with uniform particle separations ~60 Å. This distance is somewhat shorter than the maximum spacing (95 Å) expected for colloids connected by rigid DNA hybrids with the selected sequences. But because of the nicks in the DNA duplex, these are not rigid hybrids and are quite flexible. It should be noted that, in principle, this is a variable that can be controlled by reducing the system from four overlapping strands to three (thereby reducing the number of nicks) or by using triplexes instead of duplexes.

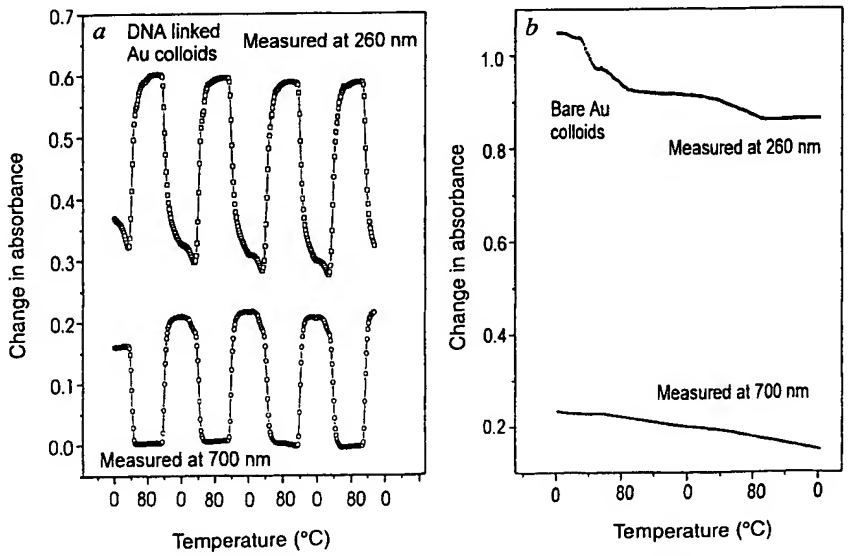

FIG. 3 *a*, Absorbance versus temperature/time profile for DNA/colloid hybridized materials. At low temperatures the Au colloids aggregate owing to the hybridization of 'linking' DNA. At high temperature (80 °C), the colloids dehybridize and form a dark red solution (see Fig. 1 and Fig. 2). The temperature versus time profile shows that this is a reversible process. *b*, Results of same procedure shown in *a*, but applied to an aqueous solution of unmodified Au colloids (5.1 nM, same concentration as in *a*).

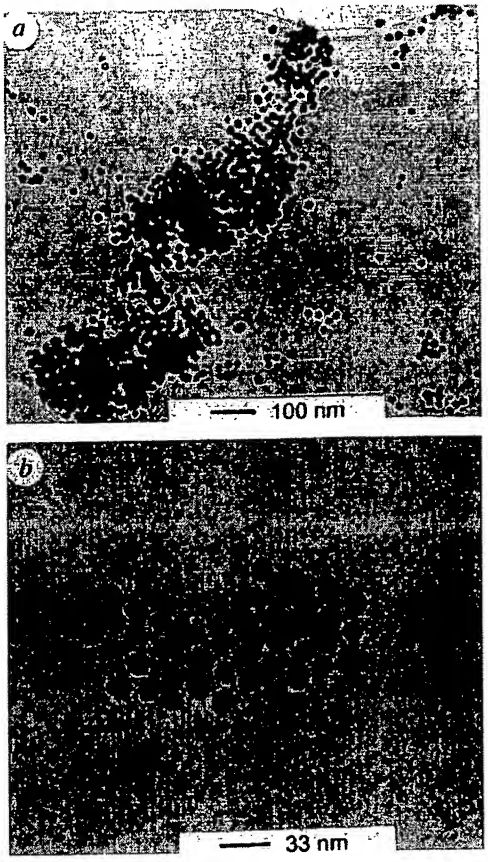

Fig. 4 TEM images of: *a*, an aggregated DNA/colloid hybrid material; *b*, a two-dimensional colloidal aggregate showing the ordering of the DNA-linked Au nanoparticles. Images were taken with a Hitachi 8100 Transmission Electron Microscope.

This work gives entry into a new class of DNA/nanoparticle hybrid materials and assemblies, which might have useful electrical, optical and structural properties that should be controllable through choice of nanoparticle size and chemical composition, and oligonucleotide sequence and length. We note that it should be possible to extend this strategy easily to other noble-metal (for example, Ag, Pt)[22] and semiconductor (for example, CdSe and CdS)[23,24] colloidal nanoparticles with well established surface coordination chemistry. Our initial results bode well for the utility of this strategy for developing new types of biosensing and sequencing schemes for DNA. The Au colloidal particles have large extinction coefficients for the bands that give rise to their colours (Fig. 2). These intense colours, which depend on particle size and concentration and interparticle distance, make these materials particularly attractive for new colorimetric sensing and sequencing strategies for DNA. □

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Organization of 'nanocrystal molecules' using DNA

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PATTERNING matter on the nanometre scale is an important objective of current materials chemistry and physics. It is driven by both the need to further miniaturize electronic components and the fact that at the nanometre scale, materials properties are strongly size-dependent and thus can be tuned sensitively[1]. In nanoscale crystals, quantum size effects and the large number of surface atoms influence the, chemical, electronic, magnetic and optical behaviour[2–4]. 'Top-down' (for example, lithographic) methods for nanoscale manipulation reach only to the upper end of the nanometre regime[5]; but whereas 'bottom-up' wet chemical techniques allow for the preparation of monodisperse, defect-free crystallites just 1–10 nm in size[6–10], ways to control the structure of nanocrystal assemblies are scarce. Here we describe a strategy for the synthesis of 'nanocrystal molecules', in which discrete numbers of gold nanocrystals are organized into spatially defined structures based on Watson-Crick base-pairing interactions. We attach single-stranded DNA oligonucleotides of defined length and sequence to individual nanocrystals, and these assemble into dimers and trimers on addition of a complementary single-stranded DNA template. We anticipate that this approach should allow the construction of more complex two- and three-dimensional assemblies.

Previous approaches towards the preparation of coupled quantum dots include co-colloids of cadmium selenide–zinc oxide (CdS–ZnO; ref. 11) and cadmium sulphide–silver iodide (CdS–AgI; ref. 12). In addition, small molecule crosslinking agents have been used to synthesize aggregates of Au (ref. 13) and cadmium sulphide linked to titanium oxide (CdS–TiO_2; ref. 14) as well as discrete dimers of cadmium selenide (CdSe; ref. 15). Finally, the collective properties of nanocrystals have been investigated using organic monolayers[16–22] and crystallization[23–26] to generate ordered arrays of inorganic quantum dots. It remains an open question whether self-assembly methods can be employed to generate complex sequences of nanocrystals.

Biological systems are characterized by remarkably complex

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LETTERS

Fabrication of CdS Nanoparticle Chains along DNA Double Strands

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Positively charged CdS nanoparticles having diameter of 3.0 ± 0.2 nm were prepared by the chemical modification of their surfaces with thiocholine. Chains of size-quantized CdS nanoparticles were prepared by using the electrostatic interaction between positively charged nanoparticle surfaces and the phosphate groups of DNA molecules. The observation by transmission electron microscopy revealed that the CdS nanoparticles were arranged in a quasi one dimension with dense packing. The line width of a nanoparticle array was equal to the diameter of CdS nanoparticles that was ca. 3.0 nm. The average distance between the centers of the adjacent nanoparticles was estimated to be 3.5 nm, which was almost equal to the length of 10 base pairs in DNA double strands.

Introduction

Fabrication of size-quantized semiconductor and metal nanoparticles arrays have attracted much attention[1−18] because of their interesting optical and electronic properties, such as photoinduced energy transfers,[2,7] metal−insulator transition,[12,13] and enhanced nonlinear optical effects.[18] A variety of strategies was performed to form nanoparticle arrays: crystallization of nanoparticles,[1−11] Langmuir−Blodgett techniques,[12−17] and chemical cross-linking between nanoparticles.[19−26] Among these methods, the last technique is not principally restricted by both size of nanoparticles and their chemical composition and seems to have advantages in order to arrange nanoparticles into a desired configuration. So far, several approaches have been reported, including the direct cross-linking between surfaces of nanoparticles with use of bifunctional molecules[19−21] and theinteraction of connecting units preattached to the surface of nanoparticles, such as the hydrogen bonding[22] and the hybridization of complementary single-stranded DNA.[23−27]

Recently a double-strand DNA molecule has been a promising construction material for fabrication of nano-structured scaffolds[28,29] because of the physicochemical stability, the linearity of molecular structure, and the mechanical rigidity.[30] For example, Au nanoparticle arrays bridged with DNA double strands have been prepared by hybridization of single-strand DNA, to which surface-modified Au nanoparticles were previously bound.[23−27] The distance between the neighboring Au nanoparticles was controlled by the length of DNA,[24−27] resulting in the exhibition of the absorption spectra characteristic of Au nanoparticles assembly. Furthermore, preparation of nanoparticles wires has been accomplished by the direct deposition of CdS[31] and Ag[32] nanoparticles on DNA double strands. As an alternative approach to prepare a wire-like structure of nanoparticle array, it may be promising to assemble the prepared nanoparticles along DNA double strands with use of an electrostatic interaction between nanoparticles and DNA

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molecules. To our best knowledge, however, such attempts have never been performed.

In this study, we have attempted to prepare CdS nanoparticle chains which have line widths equal to the size of nanoparticles, by using the electrostatic interaction between the cationic surface modifiers on the CdS nanoparticles and the phosphate groups in DNA double strands as a template.

Experimental Section

Thiocholine iodide ((2-mercaptoethyl)trimethylammonium iodide) as a cationic thiol compound was prepared by hydrolysis of *S*-acetylthiocholine iodide under acidic condition.[33,34] Acetylthiocholine iodide was added to 2.0 mol dm^{-3} HCl aqueous solution under N_2 atmosphere and stirred for 12 h, followed by neutralization with addition of 28 wt % NH_3 aqueous solution. The resulting solution which contained 0.86 mol dm^{-3} thiocholine was immediately used for the surface modification of CdS nanoparticles.

CdS nanoparticles having positive charges on their surfaces were prepared by the inverse micelle techniques under N_2 atmosphere.[35] The inverse micelles were prepared by adding 4.0 cm^3 of water into 200 cm^3 of heptane containing 14.0 g sodium bis(2-ethylhexyl)sulfosuccinate (AOT). Aqueous solutions of 1.0 mol dm^{-3} $Cd(ClO_4)_2$ (0.48 cm^3) and 1.0 mol dm^{-3} Na_2S (0.32 cm^3) were, respectively, added to 120 and 80 cm^3 aliquots of the prepared inverse micelle solution. After being stirred individually for 1 h, they were mixed together and stirred for another 1 h, resulting in the formation of CdS nanoparticles in the inverse micelles. A sample of 0.47 cm^3 of the above-mentioned thiocholine solution was added to the inverse micelle solution containing CdS nanoparticles, followed by stirring for 15 h to modify chemically the surfaces of the CdS nanoparticles with thiocholine molecules. Then, methanol was added to destroy the inverse micelles, resulting in precipitation of thiocholine-modified CdS nanoparticles. The obtained precipitate was filtered and successively washed with heptane and ethanol.

The crude CdS nanoparticles which should contain AOT as an impurity could not be dissolved in pure water, probably due to the electrostatic binding of the sulfonate groups of AOT molecules to the quaternary ammonium groups bound on the CdS surfaces. So the CdS nanoparticles were suspended in 5 cm^3 of an NaCl-saturated aqueous solution, and the solution was stirred for 1 h to exchange AOT bound on nanoparticles with chloride ions, giving a transparent yellow solution with a little amount of undissolved residue. After removing the residue by filtration, the thiocholine-modified CdS nanoparticles were subjected to a size-selective precipitation process[36] using pure water/2-propanol as a pair of solvent/nonsolvent, to narrow the particle-size distribution and to remove impurities. 2-Propanol was added to the CdS nanoparticle colloid solution until the precipitation of CdS nanoparticles appeared. The precipitate was separated by centrifugation and washed by alcohol, and then redissolved in pure water. These procedures were repeated several times. Finally, thiocholine-modified CdS nanoparticle aqueous solution was subjected to ultrafiltration to remove residual impurities by using a 2-nm pore size ultrafilter (Amicon PM-10).

Figure 1 shows schematic illustrations presenting the procedures for preparation of CdS nanoparticle arrays along DNA double strands. A chloroform solution containing both 0.33 mmol dm^{-3} dioleoyl trimethylammonium propane (DOTAP, a cationic amphiphile) (Avanti Polar Lipids) and 0.67 mmol dm^{-3} β-oleoyl-γ-palmitoyl L-α-phosphatidylcholine (OPPC, a zwitterionic amphiphile) (Wako Pure Chemicals) was prepared. This solution was spread over 20 mmol dm^{-3} Tris buffer (pH 7.0) containing 0.5 μmol dm^{-3} (base pair) salmon testes DNA (Sigma) in a Teflon trough at 27 °C to form an amphiphile monolayer having cationic net charges at air–water interface. The surface area was compressed by moving the Teflon-coated barrier until the surface pressure became 10 mN m^{-1}, and the barrier was held for 5 h to deposit DNA double strands with electrostatic interactions between the phosphate groups of DNA molecules and quaternary ammonium groups of DOTAP in the amphiphile monolayer.[37–39]

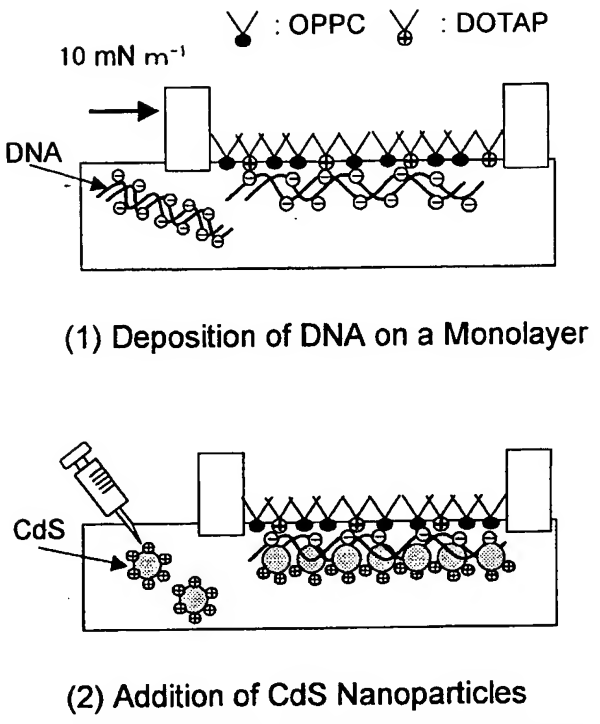

Figure 1. Schematic illustration of the deposition of a CdS nanoparticle chain along a DNA molecule on a DOTAP–OPPC mixture monolayer at air–water interface by using electrostatic interactions.

The thiocholine-modified CdS nanoparticle colloid was gently added in the water subphase so as to give 1.2×10^{17} particle dm^{-3} CdS nanoparticles at the constant surface pressure of 10 mN m^{-1}. This situation gave four CdS nanoparticles per 10 base pairs of DNA. This amount of CdS nanoparticles was four times greater than the experimentally obtained amount for densely packed CdS nanoparticle arrays along DNA double strands, as described below. The trough was left to stand for 2 h to induce electrostatic deposition of CdS nanoparticles along DNA double strands. Specimens observed by a transmission electron microscope (TEM) were prepared by transferring the DOTAP–OPPC mixture monolayer onto a copper TEM grid with an amorphous carbon overlayer. For this purpose, the TEM grid was vertically dipped through the monolayer at the air–water interface and then lifted up at 5.0 mm min^{-1}. During the deposition procedures, the surface pressure of the monolayer was kept at 23 mN m^{-1} in order to obtain the densely packed amphiphile monolayer on the TEM grid.

Results and Discussion

Thiocholine-modified CdS nanoparticles had an absorption peak due to the first exciton transition at 420 nm and an absorption onset at 470 nm as shown in Figure 2a. It is noteworthy that the obtained CdS nanoparticles exhibited the

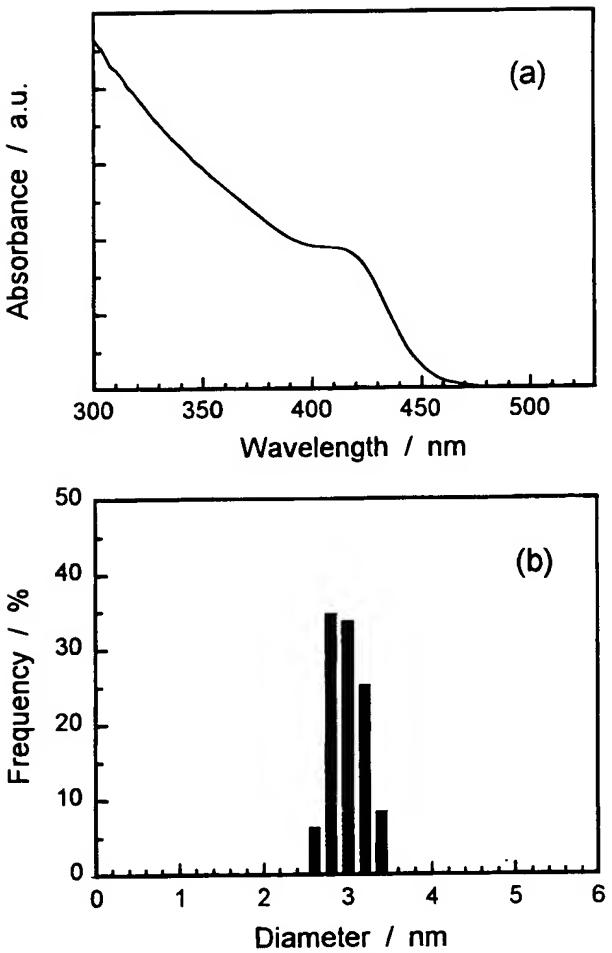

Figure 2. Absorption spectrum of thiocholine-modified CdS nanoparticles in aqueous solution (a), and their size distribution determined by TEM observations (b).

large size quantization effects since the bulk CdS particles show absorption onset at 520 nm. Figure 2b shows the size distribution of thiocholine-modified CdS nanoparticles obtained by TEM measurements. The observed CdS nanoparticles were almost spherical in shape. The average diameter was estimated to be 3.0 nm with a standard deviation of 0.20 nm. The obtained size distribution, which was 6.7% of the average diameter, was narrow enough to call the prepared CdS nanoparticles as nearly monodisperse particles. The electron diffraction patterns simultaneously obtained in the TEM measurements showed clearly only four diffraction rings corresponding to the interplanar spacings of 3.33, 2.06, 1.77, and 1.19 Å, which were assignable to diffractions from (111), (220), (311), and (422) planes of a cubic crystal structure of CdS, respectively.

Figure 3 shows surface pressure (π) − surface area (A) isotherms of amphiphiles spread on water subphase. As shown by a curve (a), the surface pressure of a DOTAP−OPPC mixture monolayer on the Tris buffer increased gradually with decrease in the area, and the monolayer collapsed at the surface pressure of 40 mN m^{-1} where the area per molecules of about 0.4 nm^2 was given. This minimum area obtained is in good agreement with the molecular cross sectional area of the dialkyl chains. The amphiphiles gave essentially the same isotherms even if the water subphase contained either DNA molecules or CdS nanoparticles as indicated by curves (b) and (c) in Figure 3, suggesting that polyion complex formation between DNA molecules and DOTAP did not influence the properties of the

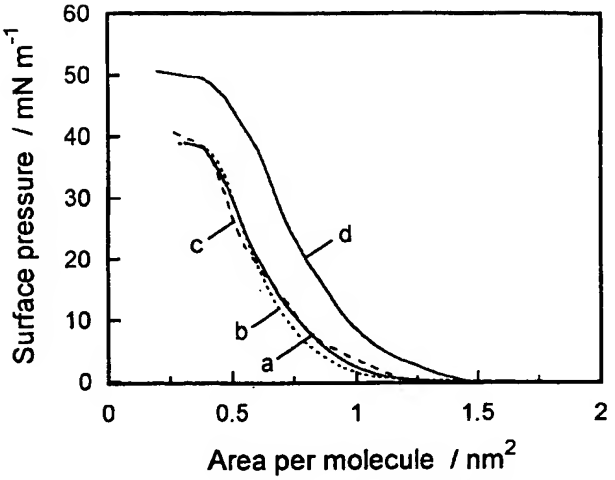

Figure 3. Surface pressure−surface area isotherms of DOTAP−OPPC (1:2) mixture monolayer on the tris buffer (pH 7) (a), and those taken on the tris buffer (pH 7) containing 0.5 μmol dm^{-3} (base pair) DNA (b), 1.2 × 10^{17} particle dm^{-3} CdS nanoparticles (c), and 0.5 μmol dm^{-3} (base pair) DNA and 1.2 × 10^{17} particle dm^{-3} CdS nanoparticles (d).

monolayer at the air−water interface, as already reported.[38] However, different behavior of $\pi-A$ isotherms was observed in the presence of DNA double strands and CdS nanoparticles together in water subphase (curve (d)). The surface area was greater at a given surface pressure, and the larger collapse pressure of 50 mN m^{-1} was obtained. These results indicated that the rigidity of DNA double strands attached on the amphiphiles monolayer would increase by the deposition of CdS nanoparticles on DNA double strands, resulting in higher stabilization of the amphiphiles monolayer.

Figure 4 shows high-resolution TEM images of amphiphiles monolayers which were transferred to the TEM grids from the water subphase. In the case of the Figure 4a, the water subphase used for the preparation of the monolayer contained only CdS nanoparticles. As recognized, there were a very small number of CdS nanoparticles and they were randomly dispersed, indicating that deposition of CdS nanoparticles on the amphiphile monolayer hardly occurred in the absence of DNA in the water subphase. On the contrary, as clearly shown in the Figure 4b, some separate chains of CdS nanoparticles were found in the amphiphiles monolayer prepared on the water subphase which contained both CdS nanoparticles and DNA double strands. Individual chains had a width of ca. 3.0 nm which accorded well with the diameter of CdS nanoparticles, suggesting that CdS nanoparticles made a single line along a DNA molecule. This was confirmed by a high magnification TEM image shown in Figure 4c. Individual CdS nanoparticles were clearly recognized, and they had the lattice fringes with the interplanar spacing of 0.33 nm assigned to the (111) plane of the cubic CdS structure. It was clearly shown that densely packed particles made a quasi-one-dimensional array without coalescence of the particles. Unlike the case of the crystallized Ag nanoparticles,[9,10] there was no correlation in the crystallographic directions between adjacent nanoparticles.

The separate CdS nanoparticle chains with dense packing were found with high reproducibility in the different amphiphile monolayers even if the concentration of DNA in the water subphase used for the preparation of the monolayer was decreased to 0.25 μmol dm^{-3} (base pair). As in the case of the preparation of the cationic liposome-bound DNA,[40] the ratio of the cationic amphiphile and the zwitterionic amphiphile in the monolayer influenced the density of the CdS nanoparticle chains

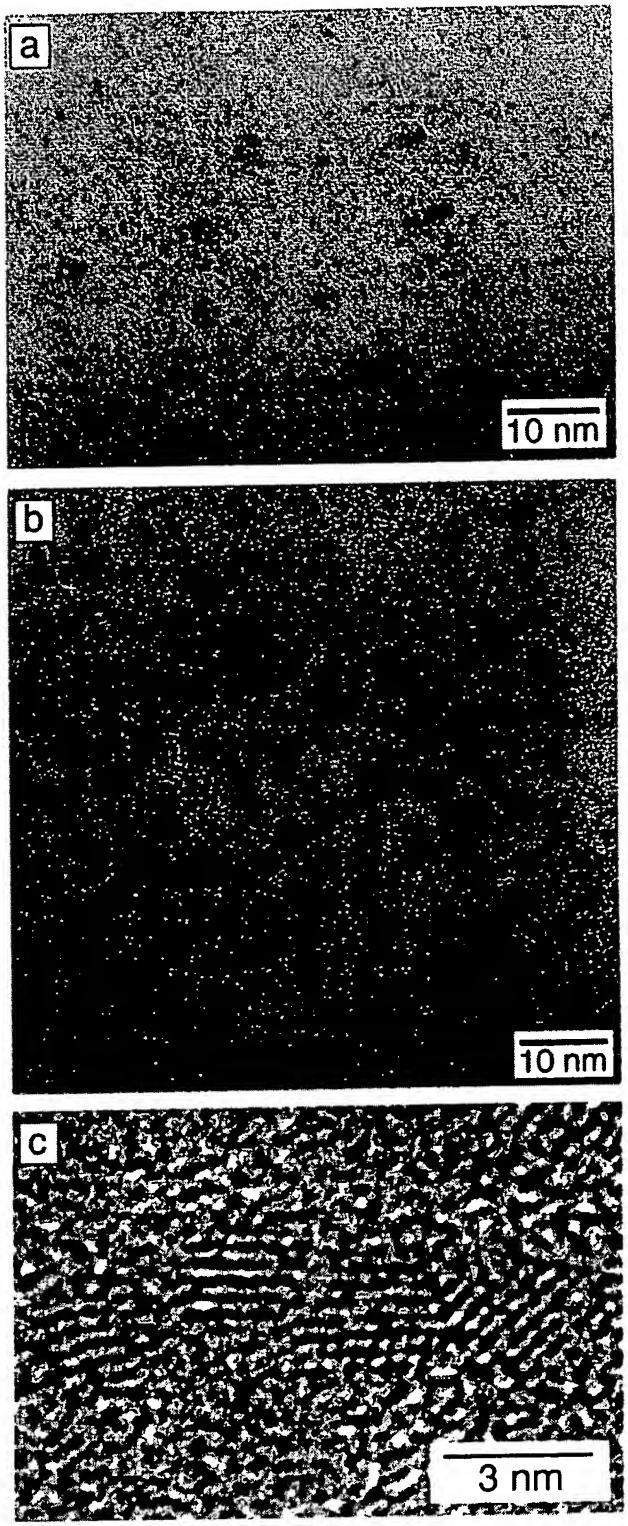

Figure 4. TEM images of the mixed monolayer of DOTAP and OPPC transferred from the air−water interface. The water subphase used contained only CdS nanoparticles (a) and both DNA double strands and CdS nanoparticles (b and c). A picture (c) is a high magnification image of CdS nanoparticles in the array shown in the picture (b).

fixed in the monolayer. If the monolayer composed of only DOTAP was used, a TEM image of the resulting monolayer showed numerous CdS nanoparticles which did not allow us to distinguish individual chains. It was concluded from the obtained results that the CdS nanoparticle chains were generated by the electrostatic attraction between negatively charged DNA molecules and positively charged nanoparticles. This was also supported by the fact that no wire-like assembly of CdS nanoparticles in the DOTAP−OPPC mixture monolayer were observed by the TEM measurements if the CdS nanoparticles surface-modified with 2-mercaptoethane sulfonate having negative charge were used.

The TEM pictures allowed us to determine the distance (d_{c-c}) between the centers of the adjacent CdS nanoparticles in the chain. The value of d_{c-c} was varied from 3 to 4.5 nm, giving the average of 3.5 nm with the standard deviation of 0.52 nm. Since the base-pair separation in DNA double strands is 0.34 nm,[30] the results suggested that a CdS nanoparticle per 10 base pairs was bound to DNA double strands. Considering that the average diameter of CdS core was 3.0 nm and the thiocholine layer modified on CdS surface was about 0.5 nm thickness, d_{c-c} was expected to be 4.0 nm in dense packing of nanoparticles. The observed difference may result from the size distribution of CdS nanoparticles and/or the interdigitation of the modified layer[41] between the nanoparticles.

Conclusion

The present study showed one successful approach to preparation of CdS nanoparticle chains by electrostatically immobilizing CdS nanoparticles along DNA double strands as a template. This methodology principally permits the fabrication of nanoparticle chains which are composed of the various nanoparticle sizes and/or the different chemical compositions. Furthermore, by combining with the techniques that have been reported to allow DNA molecules to be immobilized and assembled on solid substrates,[29,32,38,42,43] CdS nanoparticle chains can be produced on a desired position having an appropriate structure. This will be useful to fabricate the optoelectronic devices with use of nanoparticles which are prepared by wet chemical processes and have a narrow size distribution, because these devices must contain the connection between the nanoparticles and electrodes.

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Characterization of quantum-confined CdS nanocrystallites stabilized by deoxyribonucleic acid (DNA)

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Abstract. The biopolymer calf thymus deoxyribonucleic acid (DNA) is employed to stabilize cadmium sulfide crystallites in the quantum confinement size regime (Q-CdS). In this work, the synthesis and characterization of these semiconductor 'quantum dots' is described. These Q-CdS clusters are easily prepared in aqueous solution at room temperature and are extremely stable (for more than 17 months when stored at 5°C). High-resolution transmission electron microscopy shows that the crystallites have an average diameter of 5.6 nm, with lattice images and diffraction patterns consistent with the zinc-blende structure of CdS. For approximately 15% of the particles, unique hollow-sphere- or hollow-hemisphere-shaped CdS structures are observed, and their presence attributed to the influence of the DNA host. Spectroscopically, these clusters show an absorption edge blue-shifted from that of the bulk, consistent with quantum confinement, and broad trap emission characteristic of an appreciable number of defect sites at the semiconductor cluster interface, apparently induced in part by the host polynucleotide. The effects of the Q-CdS clusters on the macroscopic properties of the DNA are illustrated by the change in intrinsic viscosity upon addition of cadmium ions and subsequent CdS formation.

1. Introduction

The study of semiconductor nanocrystallites with a particle diameter comparable to the size of the bulk exciton, so-called 'quantum dots', is of current interest from the diverse perspectives of chemistry, physics, and materials science [1–4]. Their size-dependent bandgap, incomplete band structure, and three-dimensional confinement of charge carriers give rise to unique photophysical properties [5–7]. Several preparative routes for the synthesis of these materials are currently employed. Each utilizes macromolecular stabilizers or surface reactions that inhibit particle growth of the crystallites. Examples of such 'arrested precipitation' reactions include the use of simple polymers [8–11], inverse micelles [12], organic capping reagents [13–15], zeolites [16, 17], biosynthesis [18, 19], Langmuir–Blodgett films [20], layered phosphonates [21], and lipid bilayer membranes [22].

The use of polynucleotides for semiconductor cluster stabilization is appealing for two main reasons. First, there is an extensive body of knowledge regarding nucleotide structure and dynamics in solution from both macro- and microscopic techniques [23]. Second, a useful feature of nucleotides as polymeric stabilizers is the possible control of nucleotide composition as well as three-dimensional structure (single-, double-, or triple-helix) at the nanoscale level. An area of fruitful research would be to exploit this knowledge of polynucleotides as a means of examining the structural influence of these stabilizers on semiconductor cluster formation. Transition metal ions, in general, can bind to nucleic acids at three possible locations [24]: (i) the anionic oxygen atoms of the outer phosphate groups; (ii) at the hydroxyl groups of the ribose sugar moieties; and (iii) at the nitrogen atoms of the purine and pyrimidine bases. Since these semiconductor particles of cadmium sulfide are prepared by initial metal ion addition to the DNA, followed by the introduction of sulfide, there exist several possible spatial regions of the DNA where the polynucleotide should possess an affinity for the semiconductor particle.

We initiated our studies with an examination of the ability of calf thymus deoxyribonucleic acid to stabilize

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semiconductor cluster formation and to control the resultant cluster properties. Calf thymus DNA has often been chosen in the past to probe interactions of transition metal species with nucleic acids [25]. We report here the details of a synthetic route to quantum-confined cadmium sulfide (Q-CdS) stabilized by DNA obtained from calf thymus, and characterization of the Q-CdS by high-resolution transmission electron microscopy (HREM) and absorption and emission spectroscopy (steady-state and time-resolved). At the macroscopic level, the effect of CdS cluster formation on the intrinsic viscosity of the DNA is also reported.

2. Experiment

2.1. Preparation of Q-CdS/calf thymus DNA

In a typical experiment, freshly prepared $Cd(ClO_4)_2 \cdot 6H_2O$ (5.0 μl of a 1 M aqueous solution, prepared from the solid; Johnson–Matthey, electronic grade) was diluted to 2 ml and purged thoroughly (for about 20 min) with nitrogen (final Cd^{2+} concentration 4×10^{-4} M). In a separate flask, fresh Na_2S (5.0 μl of a 1 M solution, prepared from the solid; 98%, Aldrich) was diluted to 5 ml and also purged thoroughly with nitrogen (final S^{2-} concentration 4×10^{-4} M). In a third flask, approximately 15 mg calf thymus DNA was dissolved in 5.0 ml distilled deionized H_2O in a 50 ml round-bottomed flask. The nucleotide is slow to dissolve, and should be allowed to stand for about 30 min, whereupon it can then be thoroughly mixed. The relative molar concentration of nucleotide was determined spectrophotometrically by employing an ε-value of 6600 $M^{-1} cm^{-1}$ for the DNA at λ_{260} [26]. The nucleotide flask was fitted with a septum and purged very slowly with nitrogen for about 20 min. To form Q-CdS clusters, the 2 ml Cd^{2+} solution was added to the nucleotide, and the mixture was purged with nitrogen for another 5 min. The 5 ml S^{2-} solution was then transferred to the reaction flask containing the nucleotide and Cd^{2+} via syringe; the near-instantaneous appearance of a yellow color was observed.

2.2. Transmission electron microscopy

High-resolution transmission electron microscopy was performed at the Center for Materials Characterization of the University of North Texas using a Hitachi H-9000 operating at 300 kV. Samples were prepared by concentrating one of the above CdS/DNA samples in a centrifuge by spinning at 42000*g* for 20 min. The denser CdS/DNA material was collected by pipet and 1–2 drop aliquots were allowed to air-dry on amorphous carbon films supported by standard copper TEM grids. HREM images were obtained using an objective lens aperture that allowed all diffracted beams with a *d*-spacing larger than ⟨400⟩ to contribute to the images. Selected-area electron diffraction pattern (SADP) analysis was used to measure the interplanar spacings.

2.3. Steady-state spectroscopy

Absorption measurements were made using either an HP 8452A diode array spectrophotometer or a Varian Cary 3 double-beam instrument. Steady-state photoluminescence (PL) measurements were recorded using a Spex Fluorolog-2 0.22 m double spectrometer. Excitation was provided via light from a 450 W Xe lamp focused into a single 0.22 m monochromator; the typical excitation wavelength was 375 nm. Emission spectra were corrected for fluctuations in photomultiplier tube response.

2.4. Viscosity measurements

Viscosity measurements were obtained with a conventional Ostwald-type viscometer and a constant-temperature bath (±0.1°C).

2.5. Time-resolved spectroscopy

For these experiments, a setup at the Center for Fast Kinetics Research at the University of Texas-Austin was used. A Coherent Antares Nd: YAG laser, mode-locked at 76 MHz and frequency-doubled to 532 nm with a KTP crystal, produced a train of pulses 13.2 ns apart with a pulsewidth of 70 ns and an average power of 2 W. This was used to pump a home-built dye laser and cavity dumper combination containing Rhodamine 6G. The dye laser was tuned to 614 nm, and a Camac Systems, Inc., Bragg cell driver was adjusted to produce a train of pulses ca 6 ps wide at a repetition rate of 1.9 MHz from the cavity dumper. The dye laser pulses were then frequency-doubled to 307 nm with a KDP crystal to provide the excitation source. UV power never exceeded 1 mW mm^{-2}. Prior to frequency doubling, a pellicle beamsplitter led about 2% of the dye beam to a photodiode, which provided the start pulse. Fluorescence photons were detected perpendicular to the excitation by a Hamamatsu R2809U-07 microchannel plate, which provided the stop pulses. Wavelength selection for the fluorescence was achieved by placing an appropriate narrow-bandpass filter between the sample and the microchannel plate. Start and stop pulses were amplified by a Phillips Scientific Model 774 amplifier before being passed to a Tennelec TC 454 constant-fraction discriminator. The discriminator outputs were led to the start and stop channels of an Ortec 457 time-to-amplitude converter, whose output was passed to a Tracor Northern TN 7200 multichannel analyser (MCA). Data from the MCA were collected by an IBM-compatible personal computer for data analysis, storage, and display.

3. Results and discussion

3.1. Synthesis/particle stability

In this work, we have employed DNA obtained from calf thymus to stabilize the formation of CdS crystallites in the size domain exhibiting quantum confinement (Q-CdS). The synthetic methodology required is straightfor-

ward and carried out in two steps: First, a sub-millimolar (10^{-4} M) aqueous solution of cadmium ions is added to a solution of DNA of about 10^{-3} M in nucleotide concentration. After thorough ebullition with nitrogen, a 10^{-4} M solution of sulfide is then added to this mixture to generate the desired CdS cluster:

$$10^{-4}\,\mathrm{M}\ Cd^{2+}(aq) + 10^{-3}\,\mathrm{M}\ \text{DNA} \longrightarrow Cd^{2+}/\text{DNA}$$
$$\downarrow\ S^{2-}(Na_2S, H_2S)$$
$$\text{Q-CdS/DNA}$$

The intense yellow color that appears instantaneously is clearly consistent with the formation of cadmium sulfide. These clusters are stable from flocculation for 1–2 weeks when stored at room temperature in the open air; if somewhat more judicious care is used for their storage (i.e., if they are stored in closed vials at 5°C), the CdS particles are stable for greater than 17 months, with *no* evidence of aggregation or formation of bulk polycrystalline material. It is also clear that a polymeric nucleotide is required for cluster stability, since analogous experiments carried out with the monomeric nucleotides adenosine triphosphate (ATP) or adenosine monophosphate (AMP) at identical concentrations/conditions to the DNA experiments result in CdS material that flocculates within 12–24 h, regardless of storage conditions (N_2 atmosphere, temperatures ranging from 5°C to −60°C).

3.2. TEM characterization

A typical transmission electron micrograph of a Q-CdS/DNA sample is presented in figure 1. The lattice planes of numerous crystalline particles are observed. The observed lattice spacings are consistent with the zinc-blende phase of cadmium sulfide. Particle size distribution analysis was performed by measuring several hundred individual particle diameters using a Houston Instruments Digitizing Tablet interfaced to an IBM-PC. A BASIC program calculates both normal and log-normal distribution statistics and histograms. The statistical data are presented in table 1. The average particle size was 5.6 nm, with a maximum of 12 nm and minimum of 2.3 nm. The normal and log-normal histograms are presented in figure 2. From these graphs, it is apparent that the particle size distribution is best described using log-normal statistics.

Confirmation of the Q-CdS as CdS with the zinc-blende structure was obtained using SADPS. Figure 3 shows a typical pattern. The calculated *d*-spacings along with the literature values for CdS with the zincblende

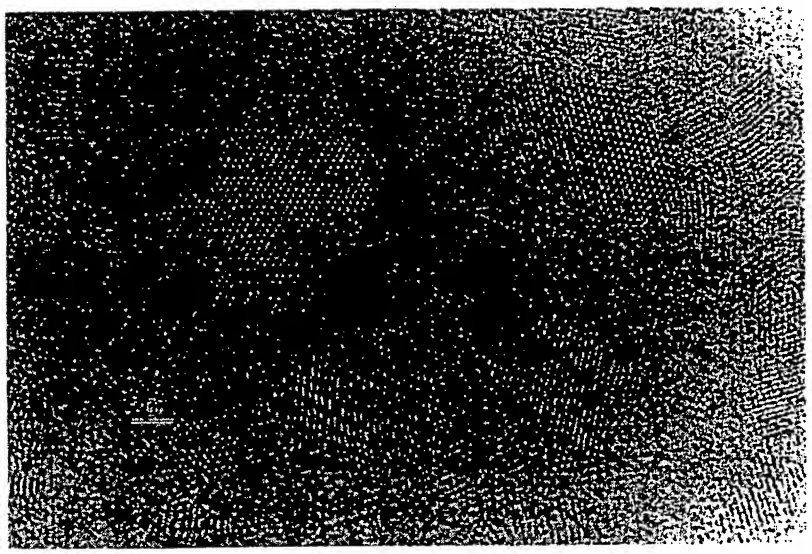

Figure 1. High-resolution TEM image of Q-CdS prepared in the presence of calf thymus DNA.

Table 1. Particle size distribution data.

Normal distribution analysis		Log-normal distribution analysis	
Average particle size	5.55 nm	Log average particle size	0.72
Standard deviation(s)	1.83 nm	Log standard deviation(s)	0.014
% standard deviation	30.98%		
Maximum particle size	12.1 nm	Log maximum particle size	1.08
Minimum particle size	2.4 nm	Log minimum particle size	0.37

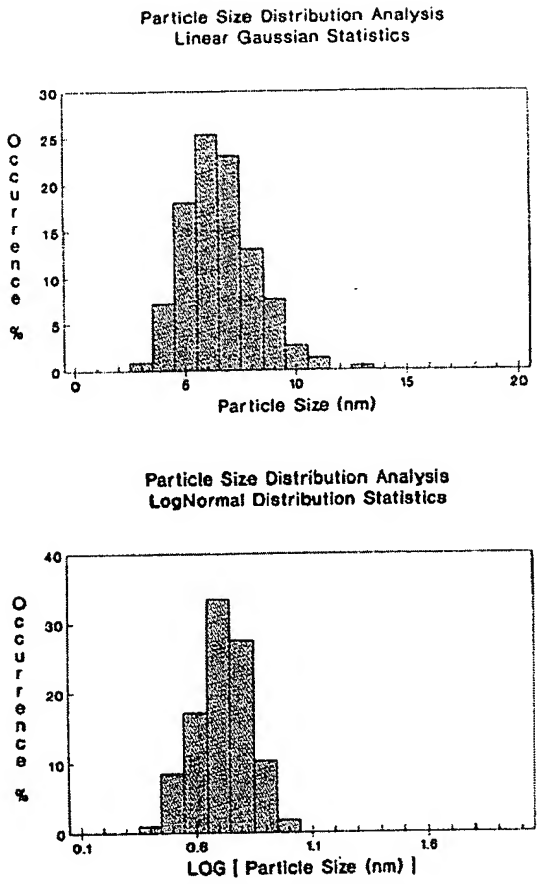

Figure 2. Particle size distribution for Q-CdS particle stabilized by calf thymus DNA.

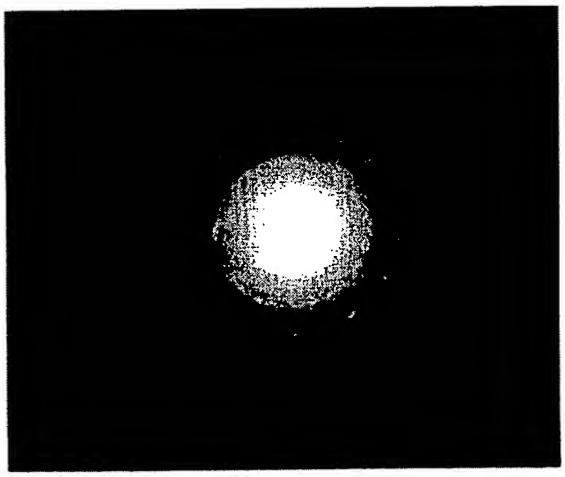

Figure 3. SAD pattern for a Q-CdS particle stabilized by calf thymus DNA.

structure, CdS with the wurtzite structure, and graphite, as published in the ICPDS files, are shown in table 2 [27]. The experimental *d*-spacings are in complete agreement with those of CdS in the zincblende form, with all of the diffraction rings present. It should be noted that the observed particles cannot be due to small graphitic inclusions in the amorphous carbon support films, since extra diffraction rings due to graphite are not seen in the SADPs, none of the observed lattice spacings in the particles are consistent with graphite, and the curved lattice planes typical of graphite are not observed.

Table 2. Comparison of observed selected area diffraction (SAD) data for Q-CdS/calf thymus DNA particles with that of ICPDS values for zinc-blende (ZB) CdS, wurtzite (W) CdS, and graphite.[a]

Experimental *d*-spacing	ZB-CdS	W-CdS	Graphite
3.30	3.30	—	3.35
—	—	3.06	—
2.83	2.86	—	—
—	—	2.65	—
—	—	—	2.13
1.99	2.02	—	2.03
—	—	1.87	—
1.71	1.72	—	1.80
1.65	1.65	1.60	1.68
1.42	1.43	—	—
	1.31	1.33	—
1.26	1.28	—	—
—	—	1.22	1.23
1.15	1.17	1.19	1.16
—	1.10	1.08	1.12

[a]All values are in angstroms (Å). The data correspond to a cubic phase with a lattice parameter $a = 5.7200$ Å.

In some cases, the centers of the particles have different contrast than the periphery (figure 4). Under certain imaging conditions, the center of a particle may lack contrast entirely and have a ring or donut shape. However, it is usually possible to find objective-lens defocusing conditions under which the lattice planes are visible across the entire particle, but with reduced contrast in the center. Approximately 15% of the particles exhibit this effect. These images may be due to particles shaped like either 'hollow spheres' or 'hollow spheres (mushroom caps)'. HREM image calculations will be performed to test this hypothesis.

Given the above observations, determination of the exact particle structure may provide information on the details of the mechanisms of particle nucleation and growth. For example, the particles may nucleate and grow while still attached to the DNA chain. Alternatively, the particles may nucleate along the biopolymer, detach, and then grow later. One possible scenario is that it is a consequence of the polynucleic acid 'template' used. As pointed out in section 1, there are three possible sites for initial Cd^{2+} ion/DNA interaction in the preparative scheme employed here. Clusters that are formed by sulfide addition to Cd^{2+} ions bound to the inner base pair region are somewhat more sterically restricted. The vast majority of clusters, however, are quasi-spherical in shape, implying formation along the periphery of the phosphate groups.

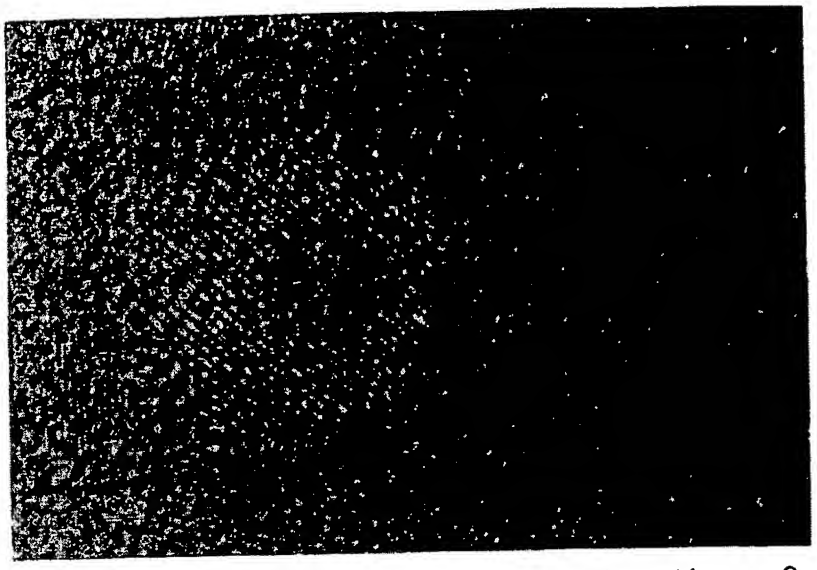

Figure 4. HREM image demonstrating the unique structure observed for some Q-CdS particles. Lattice fringes from other typical spherical particles can be seen surrounding this unique structure.

3.3. Spectroscopic properties

Optical absorption, photoluminescence excitation (PLE), and photoluminescence (PL) spectra for a representative sample of Q-CdS prepared at a concentration of 4×10^{-4} M Cd and S and 2.5×10^{-3} M nucleotide are illustrated in figure 5.

The observed absorption threshold at about 480 nm is clearly blue-shifted from that of bulk CdS (510 nm), consistent with retention of quantum confinement in the cluster [1]. No distinct higher-energy transitions, such as the occasionally observed $1S_h-1S_e$ [28], are detected in this particular case. This is due most likely to linewidth broadening effects resulting from a distribution of Q-CdS particle sizes for this preparation.

A narrower band can be detected, however, in the corresponding PLE spectrum of Q-CdS/calf thymus DNA monitored at 612 nm. By monitoring a single emission wavelength, a narrower distribution of crystallites is excited (as contrasted with the absorption experiment) and hence a sharper excitation maximum is observed at 400 nm. This maximum is attributed to the 1S–1S 'exciton' state.

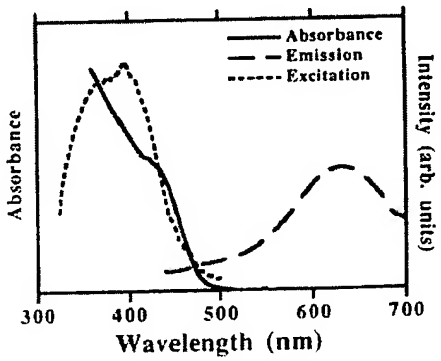

Figure 5. Optical absorption, photoluminescence excitation (PLE), and photoluminescence (PL) spectra for a representative Q-CdS sample prepared at a concentration of 4.2×10^{-4} M Cd and 2.5×10^{-3} M nucleotide. PLE spectra were monitored at 612 nm, while the PL exciting wavelength was 375 nm.

Finally, the PL spectrum of Q-CdS/DNA, also shown in figure 5, reveals broad trap emission ranging from 480–720 nm, with slight dominance of intensity near 620 nm. Earlier studies for Q-CdS have attributed emission in the 500–600 nm region as cadmium-related (V^0_{Cd}) [16, 29–31], while emission greater than 600 nm is related to sulfur vacancies (V_S^+), a specific type of this defect being Cd^{2+} centers at these sites on the particle surface [32–35]. For most existing Q-CdS preparations such as those employing inverse micelle stabilizers, 1:1 cadmium-to-sulfur ratios typically yield weakly emissive samples [12]. The presence of such strong emission observed for 1:1 Cd/S prepared in the presence of calf thymus DNA is significant, and suggests that the host polynucleotide is responsible for the particular surface photophysics observed here.

In order to probe the chemical origin of these defects further, we have examined the effects of varying cadmium ion concentration during particle formation while holding sulfide concentration constant, and vice versa. The results are illustrated in figure 6. Identical absorption spectra are obtained during the course of these experiments, consistent with indistinguishable particle size distributions. In figure 6(*a*), the effect of increasing Cd^{2+} during initial particle formation results in increased emission intensity overall, most notably at 620 nm. Such an observation is consistent with the above interpretation that emission in this region is related to sulfur vacancies, since presumably the excess cadmium ions show up as defects at the semiconductor cluster surface (a

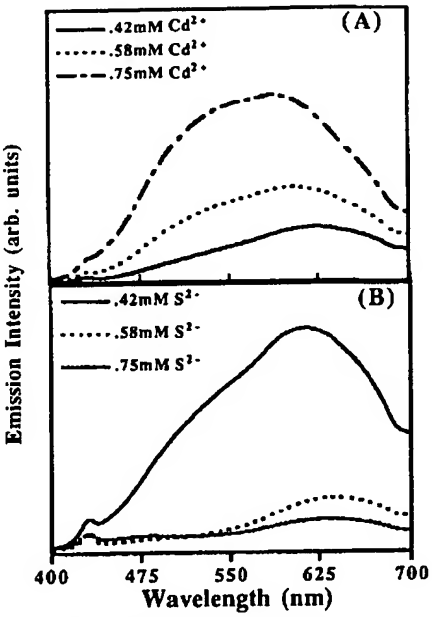

Figure 6. Emission spectra of Q-CdS solutions stabilized in 2.5×10^{-3} M calf thymus DNA, demonstrating (*a*) the effect of varying $[Cd^{2+}]$ while holding $[S^{2-}]$ constant at 4.2×10^{-4} M and (*b*) the effect of varying $[S^{2-}]$ while holding $[Cd^{2+}]$ constant at 4.2×10^{-4} M.

phenomenon observed previously for Q-CdS synthesized in inverse micelles [12, 13]). Correspondingly, the presence of excess sulfide (figure 6(*b*)) results in a diminution of emission intensity in the 500–600 nm region, while that greater than 600 nm sharpens considerably. The presence of the excess sulfide during cluster formation appears, then, to interact with Cd atoms responsible for the 550 nm emission. Such behavior is analogous to the quenching of 580 nm emission of Q-CdS in zeolites observed previously with the addition of excess H_2S [16]. This emission has also been attributed to cadmium atom defect sites in the cluster.

For both types of cluster samples (excess Cd or S), emission intensity grows with time. The linewidth of emission for Q-CdS prepared with excess sulfide narrows most markedly upon ageing in a closed vial at 5°C (FWHM shrinks by 35% in 5.5 weeks), suggesting that under these particular conditions the semiconductor surface reconstructs to yield a slightly narrower range of defect sites (traps).

The nature of these photoexcited charge carriers was further examined by monitoring the decay of the Q-CdS/DNA emission at 580 and 640 nm in the nanosecond time regime, using time-correlated single-photon counting techniques. The results of this study are illustrated in figure 7. From a visual examination of the decay curves, it is clear that the 640 nm emission is considerably slower in its decay when compared with the 580 nm emission. The experimental PL decay curves were reconvolved with the instrument response functions and then fitted to a modified version of the Kohlrausch/Williams–Watts function, which combines a single-exponential term with a stretched-exponential term:

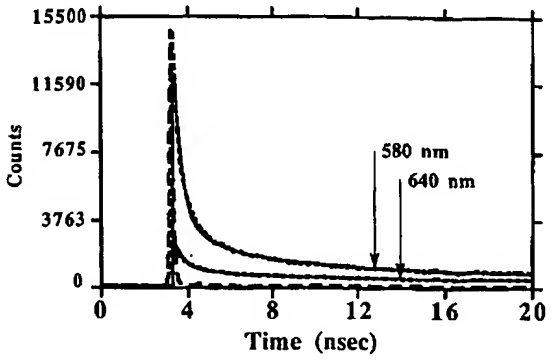

Figure 7. Decay of Q-CdS/DNA emission monitored at 580 and 640 nm, along with the corresponding fits to the data according to equation (1). A typical instrument response function is shown as a dashed curve.

$$I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)^{\beta} \qquad (1)$$

McLendon and co-workers [36] have utilized this function, both for its simplicity and its qualitative resemblance to the experimental data, to measure PL decay in Q-CdS and Q-Cd_3As_2. We note that our attempts to use a simple double-exponential function were insufficient to yield satisfactory fits of the data obtained herein, which is also consistent with previous observations. The parameter $\beta (0 < \beta < 1)$ in the stretched-exponential term is inversely related to the distribution of decay times present (as β becomes smaller, the distribution of decay times becomes broader). The parameter τ_2 is therefore only the maximum in a distribution of decay times, and the following method of Lindsay and Patterson [37] can be used to extract the average decay time $\langle \tau_2 \rangle$ from the stretched-exponential term:

$$\langle \tau_2 \rangle = \tau_2 \Gamma(\beta^{-1})/\beta \qquad (2)$$

where Γ is the gamma function, and τ_2 and β are the corresponding parameters in the stretched-exponential term. Although the quality of the fit of the 580 nm decay curve is not as good as that of the corresponding 640 nm curve (χ^2 of 2.3 versus 6.5), a comparison of the values obtained for $\langle \tau_2 \rangle$ from the two curve fits reveals that those of the longer-wavelength emission are nearly an order of magnitude greater (table 3). This result is consistent with the qualitatively observed slower decay curve of the 640 nm emission. This observation that shorter average lifetimes occur at shorter wavelengths has been observed previously for Q-CdS; it is noted to be a characteristic signature of donor–acceptor emission and reflects a significant coulombic interaction between traps [29, 33]. This view of electron hole–pair recombination in CdS/DNA cluster materials is consistent with previous studies of Q-CdS emission stabilized by other media, reflecting simultaneous radiative and nonradiative tunneling between deeply trapped carriers with strong lattice phonon participation [33]. It should be emphasized, however, that the dominant mechanism operative in the charge carrier recombination of these CdS clusters at room temperature is clearly nonradiative.

Table 3. Time-resolved photoluminescence results for Q-CdS prepared in the presence of calf thymus DNA.[a]

	580 nm	640 nm
τ_1	0.33	0.45
$\langle\tau_2\rangle$	3.92	33.3
β	0.23	0.18
χ^2	6.5	2.3

[a]The parameters listed were extracted from PL decay curves using the fitting function given in equation (1). All τ-values are in ns. $\langle\tau_2\rangle$ values were calculated using equation (2).

3.4. Cluster effects on DNA viscosity

A natural complement to the above studies that analyze the effect of the DNA on the semiconductor cluster is just the inverse; i.e., probing the effects of the cluster on the polynucleotide. In this context, and at the macroscopic level, previous measurements have illustrated the ability of viscosity to detect gross changes in DNA duplex structure. Such changes are typically brought about by some sensitive environmental perturbation such as pH, nucleotide concentration, or metal ion addition, [38]. For meaningful comparisons to be made, one must utilize the intrinsic viscosity $[\eta]$, defined as

$$\eta_{sp}/P_0 = [\eta]\,(1 - kP_0[\eta]) \qquad (3)$$

where $\eta_{sp} = (\eta - \eta_0)\eta_0^{-1}$, P_0 is the molar concentration of nucleotide (mol l^{-1}), and k is Huggins' coefficient (usually 0.5) [39].

At molar nucleotide concentrations of 10^{-3}, the highly polymerized calf thymus DNA solutions are quite viscous, with $[\eta]$-values of the order of 568 l mol^{-1}. As expected, addition of a 10^{-4} M solution of Cd^{2+} ions results in an average lowering of this value by 12.5% to 497 l mol^{-1}. A lowering of polynucleotide viscosity has been observed previously for this and other polarizable transition metal ions, and is attributed to metal binding primarily to base pairs with some disruption of hydrogen bonding (i.e., a destabilizing of the helix, reflecting a lower axial ratio) [40]. Interestingly, subsequent formation of Q-CdS clusters by sulfide addition to the above DNA/Cd^{2+} solution reveals that cluster formation forces the viscosity $[\eta]$ to increase by approximately 10% to an average value of 548 l mol^{-1}. We attribute this increased viscosity to the nucleotide strands which now strongly interact with the semiconductor surface (see above), causing a gross distortion of the DNA structure in solution as it is influenced by the large (56 Å) quasi-spherical cluster particles. In terms of viscosity, a large increase in axial ratio is consistent with a 'balling up' of the DNA as the polynucleotide wraps itself around the cluster surface. Overall, these macroscopic observations mirror the picture of an intimate CdS cluster/DNA interaction obtained from the microscopic techniques of spectroscopy and microscopy.

4. Conclusions

We have demonstrated a number of significant features regarding the ability of calf thymus DNA to stabilize the formation of quantum-confined crystallites of cadmium sulfide (Q-CdS). The semiconductor material formed is extremely stable under reasonable storage conditions (5°C). High-resolution electron microscopy unambiguously demonstrates that the material is of zinc-blende form and of average diameter within the regime of quantum confinement, with some unique structural features apparently induced by the host polynucleotide. Absorption spectroscopy confirms this quantum confinement in terms of the blue shift of its absorption edge; emission spectroscopy reveals that the CdS surface is full of defects, induced in part by the significant interaction with the DNA. The decay of this trap photoluminescence is consistent with its characterization as donor–acceptor emission. A complementary examination of the effects of the cluster on the polynucleotide viscosity present a picture of a strong CdS/DNA interaction, consistent with the other measurements in this work.

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